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Genomic Diversity of *Burkholderia pseudomallei* Isolates, Colombia

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We report an analysis of the genomic diversity of isolates of *Burkholderia pseudomallei*, the cause of melioidosis, recovered in Colombia from routine surveillance during 2016–2017. *B. pseudomallei* appears genetically diverse, suggesting it is well established and has spread across the region.

Melioidosis is caused by the environmental bacterium *Burkholderia pseudomallei*. Infections are acquired by direct contact with the pathogen, most commonly through traumatic inoculation with contaminated soil or water but also by ingestion or inhalation. Symptoms are nonspecific and can include pneumonia, skin lesions, abscess formation, and sepsis (*1*).

In Latin America, melioidosis is believed to be underdiagnosed because of the absence of reliable surveillance and the lack of available diagnostic tools and methods (*2*). Colombia has previously reported cases as sporadic, isolated events in a few geographic areas (*2*,*3*). The aim of this study was to genetically characterize isolates of *B. pseudomallei* recovered from clinical specimens in different departments of Colombia (*4*). (A department in Colombia is a geographic unit composed of municipalities led by a governor.) The goal was to better understand genetic relationships among the isolates from Colombia, as well as their relationships to isolates from other tropical and subtropical regions of the Americas. The study was internally reviewed at the US Centers for Disease

Control and Prevention (Atlanta, GA, USA) and determined not to involve human subject research.

Melioidosis is not an officially reportable disease in Colombia, but when cases are identified, department public health laboratories are required to send isolates of *B. pseudomallei* to the Instituto Nacional de Salud. During 2016–2017, a total of 11 isolates of *B. pseudomallei* were recovered from 10 melioidosis patients in the departments of Cesar (n = 4 isolates), Antioquia (n = 4), Casanare (n = 2), and Santander (n = 1) (Appendix, https://wwwnc.cdc.gov/EID/article/27/2/20-2824-App1.pdf). The most common risk factor was diabetes mellitus (n = 6); 4 of the patients died (Table). Cesar, Antioquia, Casanare, and Santander vary in population from a few hundred thousand to >6 million (*4*).

We performed whole-genome sequencing of the 11 isolates and deposited sequences at the National Center for Biotechnology Information under BioProject PRJNA638548. Sequences were used for multilocus sequence typing and single-nucleotide polymorphism (SNP) analysis (Appendix). The multilocus sequence types (ST) we observed were ones previously described, such as ST92, ST349, ST518, and ST1459. Two novel STs from this study were designated ST463 and ST1701. Previous entries in the PubMLST database (http://pubmlst.org) indicate that ST92 has been identified in cases associated with Puerto Rico and Brazil and in 1 person in Switzerland who had travelled to Martinique. ST349 was represented in 2 examples, one from Martinique and the other in a person from Spain who had travelled to West Africa; ST518 is represented in 4 examples. The first was in a person from Arizona, USA, in whom melioidosis developed after sustaining an injury while swimming in Costa Rica (*5*). In addition, ST518 was identified in *B. pseudomallei* isolates from 3 pet green iguanas, 2 of them in California, USA, and 1 in Belgium, all of which were presumably imported from Central or South America (*6*,*7*). ST1459 was noted in 1 isolate from Brazil.

SNP analysis determined from the whole genome sequences indicates that the Colombia isolates (N=11) are within the clade associated with Western Hemisphere *B. pseudomallei* based on a comparison with a panel of reference genomes (N=45) (Figure). Within this clade, a subgroup was resolved containing the Colombia genomes along with ones from Brazil and Guatemala. Also included is a genome from an isolate from a patient who had traveled to both Panama and Peru, as well as isolates from iguanas from California and Belgium, as noted, plus 1 from the Czech Republic that were presumably imported from Central or South America (Figure) (*6*–*8*).

The full panel (N = 56) was also used for quantifying SNP differences among the genomes. Patient isolates B107 and B108 had no SNPs between them, even though they were from different patients, suggesting a common source of infection or a clonal population of *B. pseudomallei* present in different sources. However, isolates B308 and B309 were from the same patient and had 1 SNP between them. The next closest relationship was for B199 (from Casanare), which diverged by 38 SNPs from B308 and by 39 SNPs from B309 (from Antioquia). The phylogenetic SNP tree indicates that isolates from Antioquia, Casanare, and Cesar for the most part do not uniformly group together by department. The largest divergence was seen between B109 and the genomes for B107 and B108, with >6,900 SNPs detected (all from Cesar). The amount of divergence plus the lack of grouping by department, even though we presume that patients' main exposures would have been within a given department, suggests *B. pseudomallei* is well established

Table. Epidemiologic and demographic characteristics of 10 melioidosis patients, Colombia

Isolate	Sequence type	Department	Age, y/sex	Type of sample	Diagnosis	Medical history and risk factors	Outcome
B107	1459	Cesar	71/M	Blood	Sepsis	Arterial hypertension	Died
B108	1459	Cesar	54/M	Right leg injury	Soft tissue infection	Tibial fracture	Recovered
B109	349	Cesar	56/M	Urine	Urinary infection	Diabetes mellitus	Recovered
B197	1463	Cesar	51/F	Bronchoalveolar lavage	Pulmonary melioidosis	Diabetes mellitus, anemic syndrome	Recovered
B198	1701	Casanare	24/M	Blood	Pneumonia	None	Died
B199	518	Casanare	26/M	Blood	Unspecified sepsis	None	Died
B255	92	Santander	68/M	Blood	Sepsis		Recovered
B308*	518	Antioquia	64/M	Tracheal aspirate	Systemic inflammatory response syndrome	Diabetes mellitus	Died
B309*				Blood			
B310	1740	Antioquia	81/F	Tracheal aspirate	Pneumonia	Kidney tumor (in studio), diabetes mellitus, arterial hypertension, hypothyroidism	Recovered
B411	1741	Antioquia	53/F	Blood	Sepsis	Diabetes mellitus	Recovered

*Isolates from the same patient.

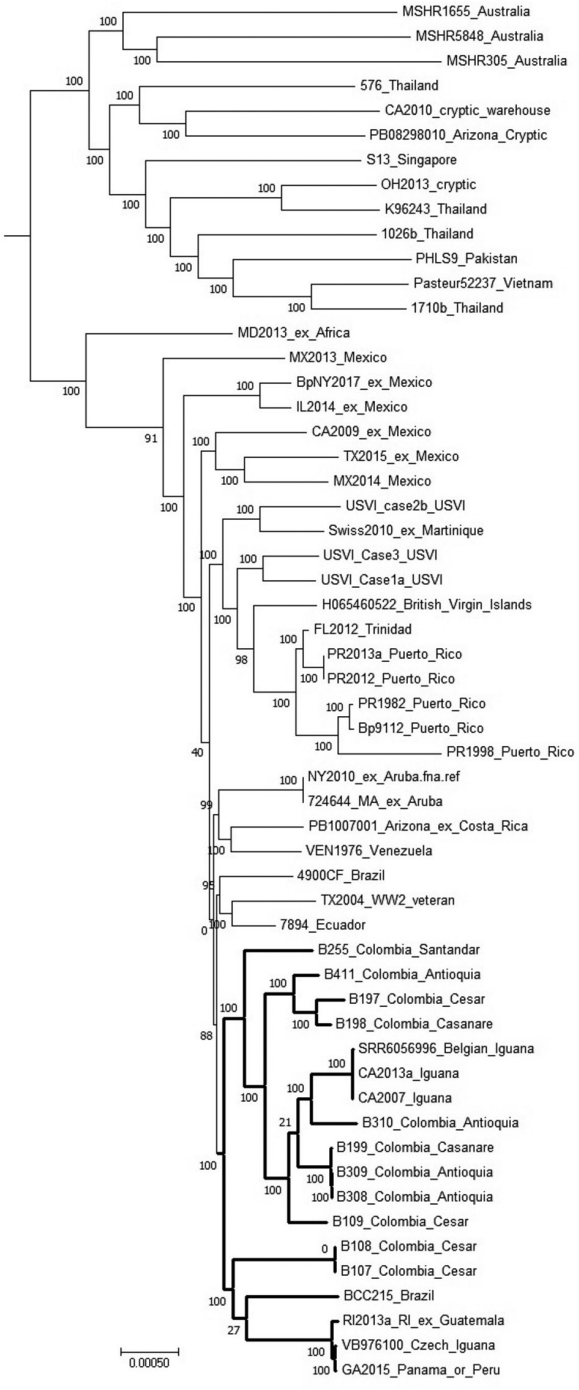

Figure. Dendrogram used for characterization of *Burkholderia pseudomallei* genomes from Colombia compared with reference genomes. Tree was generated in MEGA7 software (http://www.megasoftware.net) from results of maximum-parsimony phylogenetic analysis of core single-nucleotide polymorphisms conducted by using Parsnp, a component of the Harvest 1.3 software suite (https://github.com). Bold branches indicate the subclade containing the examples from Colombia along with reference genomes that group with them. Isolates from Colombia also include the department where they originated. Scale bar indicates number of substitutions per single nucleotide polymorphism.

in Colombia and has had time to diverge substantially since its introduction. In addition, the genomes from the 2 cases of melioidosis from pet iguanas from California and the 1 from Belgium cluster together with examples from Colombia, suggesting this region or a nearby region may have been the origin of the iguanas. Further studies, especially to recover and test environmental isolates, will improve our understanding of the population structure of *B. pseudomallei* in Colombia and improve the ability of public health stakeholders to respond to cases of melioidosis.

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Puumala Virus Infection in Family, Switzerland

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We report 3 cases of Puumala virus infection in a family in Switzerland in January 2019. Clinical manifestations of the infection ranged from mild influenza-like illness to fatal disease. This cluster illustrates the wide range of clinical manifestations of Old World hantavirus infections and the challenge of diagnosing travel-related hemorrhagic fevers.

Puumala orthohantavirus (PUUV), a species of the genus *Orthohantavirus* within the *Hantaviridae* family, is an enveloped single-strand negative-sense RNA virus (*1*). The case-fatality ratio of Old World hantaviruses ranges from 1%–10% for Dobrava-Belgrade and Hantaan orthohantaviruses to <1% for PUUV. Infection is transmitted by direct inhalation of virion-containing aerosols from rodent urine and feces. PUUV causes nephropathia epidemica, a limited form of hemorrhagic fever with renal syndrome (*1*). In Russia, 6,000–8,000 cases of hemorrhagic fever with renal syndrome are reported annually. Most cases occur in Western Russia and are caused by PUUV and Dobrava-Belgrade orthohantaviruses (*2*).

Asthenia, fever, chills, diffuse myalgia, and lumbar pain developed in a man 45 years of age 4 days after he returned to Switzerland from Samara, his hometown in central Russia (Appendix, https://wwwnc.cdc.gov/EID/article/27/2/20-3770-App1.pdf). Four days later, he sought treatment at the Geneva University Hospitals (Geneva, Switzerland) for septic shock with disseminated intravascular coagulation and kidney and liver failure. He had severe thrombocytopenia and elevated levels of C-reactive protein, procalcitonin, and leukocytes (Appendix Table 2). We transferred him to the intensive care unit for mechanical ventilation and hemodynamic support because of severe metabolic acidosis and confusion. We began treatment with broad-spectrum antimicrobial drugs, including doxycycline for possible leptospirosis. The day after admission, the patient tested positive for PUUV by real-time reverse transcription PCR (*3*) with a cycle threshold of 28. His serum sample tested positive for IgM and IgG against hantaviruses (Appendix Table 1). Shortly after his diagnosis, we administered 2 doses of 30 mg subcutaneous icatibant 6 hours apart. The patient died of multiple organ failure ≤60 hours after admission.

The next day, fever, lymphopenia, moderate thrombocytopenia, and hepatitis developed in the index patient's daughter, who was 12 years of age (Appendix). She was hospitalized and tested positive for PUUV by PCR with a cycle threshold of 26. We prescribed a 5-day course of oral ribavirin starting with an initial dose of 30 mg/kg followed by 15 mg/kg every 6 hours (*4*). The viral load in plasma rapidly decreased. We did not detect viral RNA in urine (Appendix Table 3). Interstitial nephropathy briefly developed and subsided; she was discharged without sequelae after 7 days.

The wife of the index patient had had influenza-like symptoms in Russia during the week before her husband's illness. Her serum sample tested positive for IgM and IgG against hantaviruses. We used a pseudovirus-based neutralization assay to confirm serologic results (Appendix Figure 1).

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Genomic Diversity of *Burkholderia pseudomallei* Isolates, Colombia

Appendix

Materials and Methods

Through laboratory-based surveillance activities, 11 *Burkholderia pseudomallei* isolates were received by the microbiology group of the Instituto Nacional de Salud in Colombia during 2016–2017. Cultures from blood, sputum, urine, abscesses, and throat swabs generated as part of routine diagnostic procedures were processed according to the protocols of the clinical laboratory of each hospital. We performed preliminary identification of isolates and susceptibility tests using a VITEK 2 (Biomerieux, https://www.biomerieux-usa.com). Isolates that we identified as *Burkholderia* spp., oxidase positive, gram-negative, and non-*Pseudomonas aeruginosa* bacteria, were further tested by MALDI-TOF MS (Bruker, https://www.bruker.com) (*1*).

Six isolates presumptively identified as *B. pseudomallei* or *Burkholderia* spp. were sent to the U.S. Centers for Disease Control and Prevention (CDC) for confirmatory testing, whole genome sequencing, and genetic analysis. DNA from an additional 5 *B. pseudomallei* isolates were also sent to CDC for sequencing and genetic analysis. Colombia has previously reported 20 cases as sporadic, isolated events in a few geographic areas. The departments with melioidosis cases from this study are noted on the map in the Appendix Figure. Accounts of previous cases of melioidosis in Colombia, including maps, have been published elsewhere (*2–10*).

We extracted DNA using the Maxwell RSC Cultured Cells DNA kit on the Promega Maxwell RSC Instrument per the manufacturer's instructions (https://www.promega.com) or extracted it using a QIAGEN DNeasy Blood & tissue kit (https://www.qiagen.com) from pure overnight culture, according to the manufacturer's instructions. We quantified DNA concentration and spectrum ratios using a ThermoFisher

Qubit v4.0 fluorometer (https://www.thermofisher.com). We eluted samples in PCR-grade water and RNase A, filtered through a 0.1 μm filter, and checked for sterility before whole genome sequencing (*11*).

We determined isolate sequences from paired-end Illumina reads which were generated on an Illumina MiSeq or iSeq 100 (https://www.illumina.com). We sheared genomic DNA to a mean size of 600 bp using a Covaris LE220 focused ultrasonicator (https://www.covaris.com). We cleaned DNA fragments with a Beckman Coulter Ampure system (https://www.beckmancoulter.com) and used them to prepare dual-indexed sequencing libraries using NEBNext Ultra library prep reagents (New England Biolabs, https://www.neb.com) and barcoding indices synthesized in the CDC Biotechnology Core Facility for the genomes run on the MiSeq. Libraries were analyzed for size and concentration, pooled, and denatured for loading onto the flow cell for cluster generation. We used 2 × 250 bp cycle paired-end sequencing kits to perform sequencing for the Illumina MiSeq. We used a Nextera Flex kit (Illumina) to produce libraries for the iSeq 100 runs, which we performed using 2 × 150 bp cycle paired-end sequencing kits. On completion, sequence reads were filtered for read quality, base called, and demultiplexed using bcl2fastq, version 2.19 (Illumina). We generated assemblies as previously described and assessed them with QUAST v5.0 (https://github.com; *12*,*13*). Features of the genome assemblies are noted in Appendix Table 1.

We submitted genomes to the *B. pseudomallei* MLST website (http://pubmlst.org/bpseudomallei) to identify the sequence types or assign new sequence type identifiers, as needed (*14*,*15*). We analyzed core SNPs for the genomes from Colombia using Parsnp in the Harvest 1.3 suite (https://github.com) along with a reference panel previously described, plus genomes associated with the Western Hemisphere that have recently become available (*11*,*16*–*19*). The Colombian genomes had an average of 3,822 SNPs in nonprotein-encoding (intergenic) positions compared with K96243; 2.1 × more SNPs were observed in genes that had no predicted amino acid changes (Appendix Table 2). The dendrogram was generated in MEGA 7 (https://www.megasoftware.net) (*20*). SNP effects of the Colombian isolates compared with the K96243 reference strain were predicted with SnpEff v4.3t (https://github.com; *21*).

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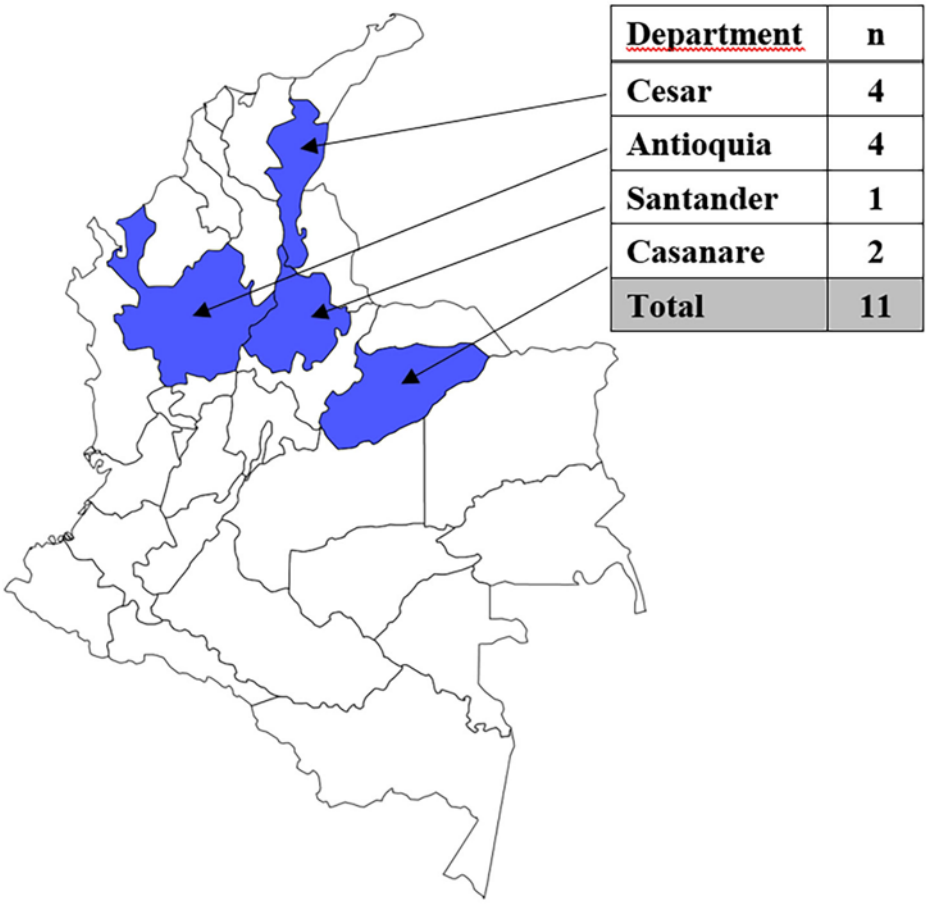
Appendix Table 1. General features of Colombian genome assemblies.

Sample	Contigs*	Total length (bp)	Largest contig, bp	GC, %†	N50‡	L50§
B107	289	7,125,249	137,454	68.08	43,818	53
B108	463	7,106,012	99,738	68.04	28,313	76
B109	444	7,134,078	134,685	68.06	31,337	68
B196	585	7,226,750	108,429	67.76	24,102	87
B197	466	7,204,391	117,090	68.01	28,036	78
B198	394	7,008,319	120,168	68.22	34,483	62
B199	259	7,040,139	292,250	68.25	51,517	39
B255	536	7,204,800	98,505	67.97	27,345	81
B308	311	7,016,254	170,164	68.24	44,603	49
B309	296	7,018,475	195,367	68.25	48,879	44
B310	321	7,086,990	152,984	68.14	40,384	52
B411	357	7,026,297	126,891	68.19	40,276	55

*No. of contiguous sequences assembled from short raw Illumina sequences
†Percentage of a genome assembly containing Guanine and Cytosine nucleotides
‡Length of the smallest contig, which together with larger contigs comprise half of the total assembly size
§Smallest contig quantity to make up 50% of the total assembly size

Appendix Table 2. Predicted mutation consequences of SNPs observed in the Colombian isolates compared with the reference strain K96243 (GCA 000959285.1).

Sample	Synonymous	Missense	Intergenic	Noncanonical start codon	Start codon lost	Stop codon gained	Stop codon lost
B107	7920	9400	3799	15	33	307	0
B108	7957	9386	3872	15	29	302	0
B109	7935	9307	3807	12	31	307	0
B196	7982	9445	3898	14	35	319	0
B197	7929	9415	3759	15	32	289	0
B198	7993	9376	3860	16	29	312	0
B199	7872	9271	3687	12	33	314	0
B255	7924	9387	3796	15	33	307	0
B308	7908	9373	3833	15	33	306	0
B309	7990	9386	3873	13	30	310	0
B310	7920	9400	3801	15	33	307	0
B411	7984	9518	3873	13	30	310	0



Department	n
Cesar	4
Antioquia	4
Santander	1
Casanare	2
Total	11

Appendix Figure. Map of Colombia showing number of melioidosis cases by department.